Docket No.: 04305/100E144-US2
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: Hans-Henrik IPSEN, et al.

Application No.: 10/719,553 Art Unit: 1644

Filed: November 20, 2004 Examiner: P. Huynh

For: **RECOMBINANT ALLERGENS**

PRELIMINARY AMENDMENT

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Prior to examination, please enter this Preliminary Amendment in the above-identified application.

Amendments to the Claims are found in the listing of claims that begins on page 2 of this paper.

Remarks begin on page 13 of this paper.

AMENDMENTS TO THE CLAIMS

The following listing of claims replaces all prior claims in the subject application.

1-35. (Cancelled).

36. (New) A recombinant mutant *Bet v 1* allergen derived from a naturally-occurring *Bet v 1* allergen, said recombinant mutant *Bet v 1* allergen having:

(a) a substitution of a solvent-accessible amino acid residue that is conserved among *Bet v 1* homologous allergens within the taxonomic order from which said naturally-occurring *Bet v 1* allergen originates, said substitution occurring in a B-cell epitope of said naturally-occurring *Bet v 1* allergen;

(b) reduced specific IgE binding compared to said naturally-occurring *Bet v 1* allergen from which it is derived; and

(c) an α-carbon backbone tertiary structure that is preserved as compared to the α-carbon backbone tertiary structure of said naturally-occurring *Bet v 1* allergen.

37. (New) The recombinant mutant *Bet v 1* allergen of claim 36 which comprises one or more amino acid substitutions selected from the group consisting of:

(i) Pro at position 10 of SEQ ID NO: 37;

(ii) Gly at position 25 of SEQ ID NO: 37;

(iii) Thr at position 28 of SEQ ID NO: 37, and Gln at position 32 of SEQ ID NO: 37;

(iv) Ser at position 45 of SEQ ID NO: 37;

(v) Ser at position 47 of SEQ ID NO: 37;

(vi) Asn at position 55 of SEQ ID NO: 37;

(vii) Ala at position 77 of SEQ ID NO: 37;

(viii) Gly at position 108 of SEQ ID NO: 37; and

(ix) Thr at position 28 of SEQ ID NO: 37, Gln at position 32 of SEQ ID NO: 37, Ser at position 45 of SEQ ID NO: 37, and Gly at position 108 of SEQ ID NO: 37.

38. (New) The recombinant mutant *Bet v 1* allergen of claim 36, wherein said solvent-accessible conserved amino acid residue has a solvent accessibility of at least 20%.

39. (New) The recombinant mutant *Bet v 1* allergen of claim 36, wherein said conserved solvent-accessible amino acid residue is conserved with more than 70% identity among *Bet v 1* homologous allergens within the taxonomic order from which said naturally-occurring *Bet v 1* allergen originates.

40. (New) The recombinant mutant *Bet v 1* allergen of claim 36, wherein the specific IgE binding of said mutant *Bet v 1* allergen compared to said naturally-occurring *Bet v 1* allergen from which it is derived is reduced by at least 5%.

41. (New) The recombinant mutant *Bet v 1* allergen of claim 36, wherein the average root mean square deviation of the atomic coordinates comparing the α-carbon backbone tertiary structures of said recombinant mutant *Bet v 1* allergen and said naturally-occurring *Bet v 1* allergen is less than 2Å.

42. (New) The recombinant mutant *Bet v 1* allergen of claim 36, wherein said conserved solvent-accessible amino acid residue is within a patch of conserved amino acid residues connected over at least 400Å of the surface of said naturally-occurring *Bet v 1* allergen.

43. (New) The recombinant mutant *Bet v 1* allergen of claim 36, wherein said solvent-accessible amino acid residue that is conserved among *Bet v 1* homologous allergens within the taxonomic order from which said naturally-occurring *Bet v 1* allergen is subsititued with an amino acid that is not conserved among *Bet v 1* homologous allergens within the taxonomic order from which said naturally-occurring *Bet v 1* allergen occurs.

44. (New) A recombinant mutant *Ves v 5* allergen derived from a naturally-occurring *Ves v 5* allergen, said recombinant mutant *Ves v 5* allergen having:

(a) a substitution of a solvent-accessible amino acid residue that is conserved among *Ves v 5* homologous allergens within the taxonomic order from which said naturally-

occurring *Ves v 5* allergen originates, said substitution occurring in a B-cell epitope of said naturally-occurring *Ves v 5* allergen;

(b) having reduced specific IgE binding compared to said naturally-occurring *Ves v 5* allergen from which it is derived; and

(c) an α-carbon backbone tertiary structure that is preserved as compared to the α-carbon backbone tertiary structure of said naturally-occurring *Ves v 5* allergen.

45. (New) The recombinant mutant *Ves v 5* allergen of claim 44 which comprises one or more amino acid substitutions selected from the group consisting of:

i) Ala at position 72 of SEQ ID NO: 39; and

ii) Ala at position 96 of SEQ ID NO: 39.

46. (New) The recombinant mutant *Ves v 5* allergen of claim 44, wherein said solvent-accessible conserved amino acid residue has a solvent accessibility of at least 20%.

47. (New) The recombinant mutant *Ves v 5* allergen of claim 44, wherein said conserved solvent-accessible amino acid residue is conserved with more than 70% identity among *Ves v 5* homologous allergens within the taxonomic order from which said naturally-occurring *Ves v 5* allergen originates.

48. (New) The recombinant mutant *Ves v 5* allergen of claim 44, wherein the specific IgE binding of said mutant *Ves v 5* allergen compared to said naturally-occurring *Ves v 5* allergen from which it is derived is reduced by at least 5%.

49. (New) The recombinant mutant *Ves v 5* allergen of claim 44, wherein the average root mean square deviation of the atomic coordinates comparing the α-carbon backbone tertiary structures of said recombinant mutant *Ves v 5* allergen and said naturally-occurring *Ves v 5* allergen is less than 2Å.

50. (New) The recombinant mutant *Ves v 5* allergen of claim 44, wherein said conserved solvent-accessible amino acid residue is within a patch of conserved amino acid residues connected over at least 400Å of the surface of said naturally-occurring *Ves v 5* allergen.

51. (New) The recombinant mutant *Ves v 5* allergen of claim 44, wherein said solvent-accessible amino acid residue that is conserved among *Ves v 5* homologous allergens within the taxonomic order from which said naturally-occurring *Ves v 5* allergen is subsititued with an amino acid that is not conserved among *Ves v 5 1* homologous allergens within the taxonomic order from which said naturally-occurring *Ves v 5* allergen occurs.

52. (New) A method of preparing a recombinant mutant *Bet v 1* allergen, which method comprises:

identifying an amino acid residue in a B-cell epitope of a naturally-occurring *Bet v 1* allergen that is conserved among homologous *Bet v 1* proteins within the taxonomic order from which said naturally-occurring *Bet v 1* allergen originates; and

substituting said identified amino acid residue to form a mutant *Bet v 1* allergen.

53. (New) The method of claim 52 further comprising determining that the α-carbon backbone tertiary structure of said mutant *Bet v 1* allergen is preserved compared with the α-carbon backbone tertiary structure of said naturally-occurring *Bet v 1* allergen.

54. (New) The method claim 52 further comprising determining that said mutant *Bet v 1* allergen has reduced specific IgE binding compared to said naturally-occurring *Bet v 1* allergen.

55. (New) The method of claim 52, wherein said substituted conserved amino acid residue has a solvent accessibility of at least 20%.

56. (New) The method of claim 52, wherein said substituted amino acid residue is conserved with more than 70% identity among *Bet v 1* homologous allergens within the taxonomic order from which said naturally-occurring *Bet v 1* allergen originates.

57. (New) The method of claim 52 wherein said identified amino acid is within a conserved patch connected over at least 400 $Å^2$ of the surface of the three-dimensional structure of said naturally-occurring *Bet v 1* allergen.

58. (New) The method of claim 52 which comprises substituting said identified amino acid residue with an amino acid that is not conserved among *Bet v 1* homologous allergens within the taxonomic order from which said naturally-occurring *Bet v 1* allergen occurs.

59. (New) A method of preparing a recombinant mutant *Ves v 5* allergen, which method comprises:

identifying an amino acid residue in a B-cell epitope of a naturally-occurring *Ves v 5* allergen that is conserved among homologous *Ves v 5* proteins within the taxonomic order from which said naturally-occurring *Ves v 5* allergen originates; and

substituting said identified amino acid residue to form a mutant *Ves v 5* allergen.

60. (New) The method of claim 59 further comprising determining that the α-carbon backbone tertiary structure of said mutant *Ves v 5* allergen is preserved compared with the α-carbon backbone tertiary structure of said naturally-occurring *Ves v 5* allergen.

61. (New) The method claim 59 further comprising determining that said mutant *Ves v 5* allergen has reduced specific IgE binding compared to said naturally-occurring *Ves v 5* allergen.

62. (New) The method of claim 59, wherein said substituted conserved amino acid residue has a solvent accessibility of at least 20%.

63. (New) The method of claim 59, wherein said substituted amino acid residue is conserved with more than 70% identity among *Ves v 5* homologous allergens within the taxonomic order from which said naturally-occurring *Ves v 5* allergen originates.

64. (New) The method of claim 59 wherein said identified amino acid is within a conserved patch connected over at least 400 $Å^2$ of the surface of the three-dimensional structure of said naturally-occurring *Ves v 5* allergen.

65. (New) The method of claim 59 which comprises substituting said identified amino acid residue with an amino acid that is not conserved among *Ves v 5* homologous allergens within the taxonomic order from which said naturally-occurring *Ves v 5* allergen occurs.

66. (New) A recombinant mutant allergen derived from a naturally-occurring allergen selected from the group consisting of (i) allergens homologous to *Bet v 1*; and (ii) vespid antigen 5 allergens,

said recombinant mutant allergen having:

(a) a substitution of a solvent-accessible amino acid residue that is conserved among homologous allergens within the taxonomic order from which said naturally-occurring allergen originates, said substitution occurring in a B-cell epitope of said naturally-occurring allergen;

(b) reduced specific IgE binding compared to said naturally-occurring allergen; and

(c) an α-carbon backbone tertiary structure that is preserved as compared to the α-carbon backbone tertiary structure of said naturally-occurring allergen.

67. (New) The recombinant allergen according to claim 66 wherein said allergens homologous to *Bet v 1* have an amino sequence that yields a BLAST probability of less than 0.1 when compared to an amino acid sequence of SEQ ID NO: 37.

68. (New) The recombinant mutant allergen of claim 66, wherein said solvent-accessible conserved amino acid residue has a solvent accessibility of at least 20%.

69. (New) The recombinant mutant allergen of claim 66, wherein said conserved solvent-accessible amino acid residue is conserved with more than 70% identity among homologous allergens within the taxonomic order from which said naturally-occurring allergen originates.

70. (New) The recombinant mutant allergen of claim 66, wherein the specific IgE binding of said mutant allergen compared to said naturally-occurring allergen from which it is derived is reduced by at least 5%.

71. (New) The recombinant mutant allergen of claim 66, wherein the average root mean square deviation of the atomic coordinates comparing the α-carbon backbone tertiary structures of said recombinant mutant allergen and said naturally-occurring allergen is less than 2Å.

72. (New) The recombinant mutant allergen of claim 66, wherein said conserved solvent-accessible amino acid residue is within a patch of conserved amino acid residues connected over at least 400Å^2 of the surface of said naturally-occurring allergen.

73. (New) The recombinant mutant allergen of claim 66, wherein said solvent-accessible amino acid residue that is conserved among homologous allergens within the taxonomic order from which said naturally-occurring allergen is subsititued with an amino acid that is not conserved among homologous allergens within the taxonomic order from which said naturally-occurring allergen occurs.

74. (New) A method of preparing a recombinant mutant allergen derived from a naturally-occurring allergen selected from the group consisting of (i) allergens homologous to *Bet v 1*; and (ii) vespid antigen 5 allergens, which method comprises:

identifying an amino acid residue in a B-cell epitope of said naturally-occurring allergen that is conserved among homologous proteins within the taxonomic order from which said naturally-occurring allergen originates; and

substituting said identified amino acid residue to form a mutant allergen.

75. (New) The method of claim 74 wherein said allergens homologous to *Bet v 1* have an amino sequence that yields a BLAST probability of less than 0.1 when compared to an amino acid sequence of SEQ ID NO: 37.

76. (New) The method of claim 74 further comprising determining that the α-carbon backbone tertiary structure of said mutant allergen is preserved compared with the α-carbon backbone tertiary structure of said naturally-occurring allergen.

77. (New) The method claim 74 further comprising determining that said mutant allergen has reduced specific IgE binding compared to said naturally-occurring allergen.

78. (New) The method of claim 74, wherein said substituted conserved amino acid residue has a solvent accessibility of at least 20%.

79. (New) The method of claim 74, wherein said substituted amino acid residue is conserved with more than 70% identity among homologous allergens within the taxonomic order from which said naturally-occurring allergen originates.

80. (New) The method of claim 74 wherein said identified amino acid is within a conserved patch connected over at least 400 $Å^2$ of the surface of the three-dimensional structure of said naturally-occurring allergen.

81. (New) The method of claim 74 which comprises substituting said identified amino acid residue with an amino acid that is not conserved among homologous allergens within the taxonomic order from which said naturally-occurring allergen occurs.

82. (New) A recombinant mutant allergen derived from a naturally-occurring allergen, said recombinant mutant allergen having:

(a) a substitution of a solvent-accessible amino acid residue that is conserved among homologous allergens within the taxonomic order from which said naturally-occurring allergen originates, said substitution occurring in a B-cell epitope of said naturally-occurring allergen;

(b) reduced specific IgE binding compared to said naturally-occurring allergen from which it is derived; and

(c) an α-carbon backbone tertiary structure that is preserved as compared to the α-carbon backbone tertiary structure of said naturally-occurring allergen.

83. (New) The recombinant mutant allergen of claim 82, wherein said solvent-accessible conserved amino acid residue has a solvent accessibility of at least 20%.

84. (New) The recombinant mutant allergen of claim 82, wherein said conserved solvent-accessible amino acid residue is conserved with more than 70% identity among homologous allergens within the taxonomic order from which said naturally-occurring allergen originates.

85. (New) The recombinant mutant allergen of claim 82, wherein the specific IgE binding of said mutant allergen compared to said naturally-occurring allergen from which it is derived is reduced by at least 5%.

86. (New) The recombinant mutant allergen of claim 82, wherein the average root mean square deviation of the atomic coordinates comparing the α-carbon backbone tertiary structures of said recombinant mutant allergen and said naturally-occurring allergen is less than 2Å.

87. (New) The recombinant mutant allergen of claim 82, wherein said conserved solvent-accessible amino acid residue is within a patch of conserved amino acid residues connected over at least 400$Å^2$ of the surface of said naturally-occurring allergen.

88. (New) The recombinant mutant allergen of claim 82, wherein said solvent-accessible amino acid residue that is conserved among homologous allergens within the taxonomic order from which said naturally-occurring allergen occurs is subsititued with an amino acid that is not conserved among homologous allergens within the taxonomic order from which said naturally-occurring allergen occurs.

89. (New) A method of preparing a recombinant mutant allergen comprising,

identifying an amino acid residue in a B-cell epitope of a naturally-occurring allergen that is conserved among homologous proteins within the taxonomic order from which said naturally-occurring allergen originates; and

substituting said identified amino acid residue to form a mutant allergen.

90. (New) The method of claim 89 further comprising determining that the α-carbon backbone tertiary structure of said mutant allergen is preserved compared with the α-carbon backbone tertiary structure of said naturally-occurring allergen.

91. (New) The method claim 89 further comprising determining that said mutant allergen has reduced specific IgE binding compared to said naturally-occurring allergen.

92. (New) The method of claim 89, wherein said substituted conserved amino acid residue has a solvent accessibility of at least 20%.

93. (New) The method of claim 89, wherein said substituted amino acid residue is conserved with more than 70% identity among homologous allergens within the taxonomic order from which said naturally-occurring allergen originates.

94. (New) The method of claim 89 wherein said identified amino acid is within a conserved patch connected over at least 400 $Å^2$ of the surface of the three-dimensional structure of said naturally-occurring allergen.

95. (New) The method of claim 89 which comprises substituting said identified amino acid residue with an amino acid that is not conserved among homologous allergens within the taxonomic order from which said naturally-occurring allergen occurs.

96. (New) A method for preparing a recombinant mutant allergen, which method comprises expressing a recombinant construct encoding a naturally occurring allergen in which an amino acid residue in a B-cell epitope of a naturally occurring allergen that is conserved among said naturally occurring allergen and homologous proteins within the taxonomic order from which said naturally occurring allergen originated has been substituted; and

purifying said mutant allergen.

REMARKS

I. Claim status. Claims 1-35 have been cancelled, without prejudice or disclaimer. New claims 36-96 have been added. Support for the new claims is found in the specification at, e.g., page 14, line 15 - page 15, line 24; page 16, line 31-page 17, line 32; page 19, lines 30-33; page 20, lines 9-29; page 26, lines 1-14; page 29, lines 5-12; page 46, line 35 - page 47, line 6; page 47, line 33 - page 48, line 23; and original claims 1-27. By this Amendment, no new matter has been added to the application.

II. Examiner's interview. On May 18, 2004, Examiners C. Chan and P. Huynh and Applicants' representatives met at the USPTO to discuss proposed claims. Applicants' representatives express their thanks to the Examiners for the courtesies extended during the interview. Although no agreement on allowable claims was reached during the interview, there was significant progress in identifying patentable subject matter. In an Interview Summary dated May 21, 2004, the Examiner indicated agreement was reached to amend certain claims by defining that a B-cell epitope is being mutated and by removing the limitation that substitutions be non-conserved amino acids. Applicants have effectively adopted these amendments in the new independent claims. The limitation that substitutions be with non-conserved amino acids has been moved to dependent claims.

Applicants provide the following summary of the issues discussed during the May 18 interview:

Allergic reaction results when allergen is recognized and bound by pre-existing IgE in a subject's plasma. Multiple IgE molecules bound to different epitopes on the allergen crosslink IgE receptors on mast cells, which in turn release histamines and other agents that cause the symptoms of allergy. In highly sensitive individuals, these mediators are released in high enough concentrations to result in anaphylactic shock, which can be fatal.

Various approaches to treating allergy involve reducing the effect of the endogenous IgE molecules. One such approach, used now for many years, involves "diluting" the endogenous IgE by eliciting a strong IgG antibody response to the allergen, by immunizing the allergic subject with the allergen. Allergy immunotherapy with the allergen is understood to work by inducing an IgG response against an allergen. The IgG produced as a result of vaccination with allergen binds to epitopes on all exposed portions of the allergen, including (or overlapping) epitopes that are bound by the pre-existing IgE. The IgG produced during

vaccination and the pre-existing IgE therefore compete for binding to the allergen. Following allergy vaccination, when a patient encounters allergen, the IgG produced as a result of vaccination competes for and reduces binding of the pre-existing IgE to the allergen, thus reducing the severity of, or inhibiting, the allergic response.

The problem with immunotherapy using the naturally occuring allergen is readily apparent: The need to avoid a severe allergic response to the therapy itself, while producing an effective allergy vaccine that generates IgG that recognizes native allergen. Native allergen is an effective antigen for use in allergy vaccines. Native allergen, however, includes the epitopes recognized by allergen patient's pre-existing IgE, leading the possibility of inducing an allergic response during the vaccination process. Allergists have tried various approaches to avoiding this problem, including using allergen fragments, denatured allergen and random mutants. The approaches tend to render the vaccine less effective, for although they dramatically reduce binding by IgE antibodies, the IgG antibodies that are produced have correspondingly low affinity for native allergen.

The present invention provides recombinant mutant allergens and methods of making them for use in allergy vaccines that reduce IgE binding but preserve enough structure to yield a strong IgG response against the native allergen. The methods described in the specification identify solvent-accessible amino acids on the allergen surface that are part of epitopes recognized by allergy patients' pre-existing IgE. Mutating one or more of these amino acids by substituting it with another amino acid residue disrupts the IgE-epitope, reducing the binding of pre-existing IgE, thus lowering the likelihood of allergic response when the recombinant allergen is used in an allergy vaccine.

As discussed above, IgEs of different epitope specificity, must find the allergen for IgE receptor crosslinking, which leads to the release of allergy mediators to occur. The elimination of even one IgE epitope can affect this crosslinking and mediator release. Destruction or reduction of the IgE-epitope, by substituting one solvent-exposed amino acid residue, however, has minimal affect on the ability of the recombinant allergen to elicit a protective IgG response, because such mutant allergens retain native three-dimensional structure. Accordingly, potential IgG epitopes spread over the surface of the allergen are preserved. Thus, the mutant allergen can still generate an effective IgG response. The IgG produced will then bind to allergen, reducing IgE binding and preventing binding to and crosslinking of the IgE

receptors, when the patient is exposed to allergen. Thus recombinant allergens produced by the methods described in the instant specification are safer and more effective antigens for use in allergy vaccines.

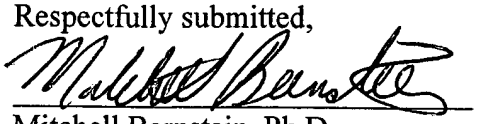
The specification describes and enables the claimed invention. Each of the steps for practicing the invention are well known to those skilled in the art. Methods of identifying and aligning homologous proteins; methods of determining three-dimensional structures of proteins and determining solvent; accessibility and techniques for showing the alpha-carbon backbone of a mutant allergen is preserved relative to the naturally occurring allergen are all well known. Moreover, the claimed invention has been reduced to practice. The specification reports on a total of seven different mutant allergens, derived from two unrelated allergens native allergens-- five independent single mutants in *Bet v 1* and two independent single mutants in *Ves v 5*. The specification also reports on an eighth mutant allergen, a triple *Bet v 1* mutant that has three of the single *Bet v 1* mutations combined in one recombinant *Bet v 1* mutant. All of the eight recombinant mutant allergens have the properties of the claimed allergens, i.e., reduced IgE binding and a preserved alpha-carbon backbone compared to native allergen. Every mutant produced had the desired properties. The claimed method can be used predictably to produce recombinant mutant allergen with the claimed characteristics. Hence, one of ordinary skill in the art can practice the claimed invention with any protein without undue experimentation.

CONCLUSION

The subject application is believed to be in condition for allowance, which is earnestly solicited. If there are any other issues remaining which the Examiner believes could be resolved through either an additional Preliminary Amendment or an Examiner's Amendment, the Examiner is respectfully requested to contact the undersigned at the telephone number indicated below.

December 21, 2004

Respectfully submitted,



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